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PLACENTATION IN THE RABBIT

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LITERATURE REVIEW

The domestic rabbit has been found to be an extremely useful animal in studies on reproduction. A mature female rabbit, maintained in isolation under optimum conditions, remains in estrus almost continuously and will breed throughout the year. Mature follicles are continuously in the ovaries, ready to ovulate under a sexual stimulus. Completion of copulation is easily marked by the buck falling sideways in a cataleptic state (Brambell, 1951). Ovulation occurs with great regularity at an interval of ten hours after copulation (Walton and Hammond, 1928). Therefore, one is able to secure embryonic material from the rabbit which can be aged to within one hour post ovulation.

After ovulation, the passage of the ova through the oviduct takes three days, during which time fertilization takes place. The zygotes reach the uterus after this time as early blastocysts where they remain free in the uterine lumen for another three days (Brambell, 1951). During this second three-day period, the blastocysts are spaced out at approximately equal intervals in the uterus and expand into spherical vesicles about 3.5 mm in diameter (Brambell, 1951; Boving, 1954).

The fact that blastocysts become evenly spaced implies that there is some sort of interplay between blastocysts and uterus (Corner, 1921). Although the number of blastocysts varies, spacing is always proportionate to their number, ruling out the possibility of predestined implantation sites (Boving, 1954). Boving (1954), in the most recent and extensive work in this field, suggested a system of spacing in terms of "a stimulus from each

blastocyst and a detector and effector system in the uterus."

After the blastocysts have reached their definitive positions in the uterus, each becomes oriented in the lumen with its embryonic shield toward the mesometrium (Brambell, 1951; Boving, 1952; Mossman, 1937). Assheton (1895) concluded that this orientation of rabbit blastocysts came about by conforming to the internal shape of the uterus, rotating until the slight flatness at the embryonic pole fitted the intrusion of the mesometrial folds. However, when suitably flattened models were inserted into the uteri of pregnant rabbits it was found that no consistent orientation was attained (Boving, 1954). He suggested that this orientation might be due to the fact that attachment between blastocyst and uterus is first attained at the abembryonic hemisphere on the antimesometrial surface. This attachment does not occur by the well known cellular invasion but by preceding adhesion involving abembryonic trophoblast, uterine epithelium, and "the intervening non-cellular blastocyst covering" (Boving, 1954). In some way not yet described, this adhesion is restricted to the membrane over the abembryonic hemisphere of the blastocyst.

Spacing and orientation of the blastocysts has been accomplished by the seventh day post coitum and on the eighth day the zona pellucida is shed, exposing the trophoblast, and the first organic contact of the embryonic and maternal tissues is established (Assheton, 1895; Brambell, 1951; Amoroso, 1952; Boving, 1954).

At this stage the endometrium of the rabbit shows the typical structures of the follicular stage of the mammalian cycle. The uterus of the rabbit has specilizations consisting of symmetrical pairs of longitudinal folds first

described by Hollard (1863) and named by Minot (1890). These folds are (1) the placental folds, the largest, situated one on each side of the groove corresponding to the insertion of the mesometrium; (2) ob-placental folds, the smallest, opposite the mesometrium, and (3) peri-placental folds, intermediate in position and size. Each placental fold is divided by transverse fissures into rectangular areas, the cousinets of Hollard (1863). These folds of the mucosa are essentially increased areas of the mucosal connective tissue, but they differ from the caruncles of Ruminants in having glands (Amoroso, 1952).

Slight localized thickenings of the "trophoblast" in the abembryonic hemisphere have been observed beginning about six and one-half days post coitum and are very prominent by the time the zona pellucida is shed on the eighth day (Mossman, 1937; Brambell, 1951; Boving, 1962). At the same time, great numbers of "giant cells" (Sansom, 1927) or "Monster Cells" (Minot, 1890) begin to appear in the uterine mucosa of the ob-placental folds (Amoroso, 1952). These giant cells are derived, according to Chipman (1903), from "degenerative hypertrophy" of the epithelium of the surface and glands of the mucosa. Hammond (1917) suggests, however, that these are detached cells from the trophoblast and therefore of fetal origin, and is supported in this view by Sansom (1927). Brambell (1951) states that they arise from the maternal vascular endothelium.

As stated earlier, the first attachment of the blastocyst to maternal tissue is on the antimesometrial surface of the uterus in the eighth day of gestation. According to Amoroso (1952), the antimesometrial uterine epithelium undergoes degenerative changes and forms a symplasm. The trophoblast in the thickened

regions invades this symplasm, forming a number of scattered fusion areas (Brambell, 1952; Mossman, 1937; Boving, 1962). Perry (1950) reported that maternal capillaries in the mucosa grow toward the invading syncytial trophoblast of the fusion areas, which envelops them and erodes their walls so that the maternal blood circulates in lacunae in the trophoblast. Boving (1962) presents experimental evidence that the sight of trophoblastic invasion is in an area which immediately overlies a maternal blood vessel. He goes on to state that trophoblastic processes penetrate the uterine epithelium and surround the immediately underlying blood vessels. The trophoblast then destroys these vessels and allows maternal blood to enter spaces within the trophoblastic syncytium.

At this stage the whole of the abembryonic hemisphere of the blastocyst beyond the sinus terminalis consists of bilaminar omphalopleur of trophoblast and entoderm only. Reichert's membrane, a non-cellular hyaline membrane developed between the trophoblast and entoderm in most mammals, is never present in the rabbit (Brambell, 1951). As the amniotic folds form, the outer trophoblast attaches to the uterine epithelium of the two mesometrial folds of the mucosa.

The antimesometrial attachments persist for only a short time after the placental attachment has begun on the mesometrial surface (Brambell, 1951). During the ninth day post coitum the superficial regions of the mucosa and the invading trophoblast of the fusion areas become disrupted (Brambell,1951). At the same time a new uterine epithelium is being regenerated from the lining of the deeper parts of the uterine glands. Regeneration is completed

by the end of the eleventh day and the giant cells which have been developing below this new epithelium form a conspicuous feature of the mucosa. The superficial layer of the mucosa, which has degenerated and beneath which the new epithelium is formed, is comparable to the decidua capsularis of rodents (Brambell, 1951). By the fourteenth day, all that remains of the bilaminar omphalopleur is a freely hanging fringe along the sinus terminalis. When the bilaminar omphalopleur degenerates, the entodermal surface of the upper hemisphere of the yolk sac follows the contour of the regenerated antimesometrial uterine epithelium, although it neither fuses with nor is in contact with the epithelium at any stage (Mossman, 1937; Brambell, 1951).

During the latter part of the eighth day the attachment of the "chorion" to the placental folds has been accomplished (Beidl, Peters and Hofstatter, 1921; Mossman, 1937; Amoroso, 1952). In the sixth and seventh days the uterine epithelium cells covering the free surface of the placental lobes proliferate and fill the superficial crypts of the mucous membrane. The glands are unchanged and the increasing blood supply leads to a secretion which is usually considered to be added to the albumen layer, subsequently to be absorbed by the trophoblast. Enlargement of the blastocyst has stretched the uterus, leveling the folds so that at the time of attachment the surfaces of the placental folds are nearly regular. Active proliferation of the connective tissue forms the "placental cotyledons". "The uterine epithelium swells and becomes syncytial as the trophoblast approaches it and is soon compressed into a thin layer between the trophoblast and connective tissue. It then undergoes degeneration and disappears, apparently being absorbed by the cells of

the trophoblast" (Amoroso, 1952). With this complete disappearance of the epithelium, the trophoblast comes into contact with the connective tissue of the uterus (Wislocki and Streeter, 1938).

The epithelium of the glands also proliferates, swells and forms a symplasma which obliterates the lumina and even dilates the glands many times beyond their original size. The uterine glands form a temporary pathway for the invasion by the trophoblast, and they eventually disappear on about the tenth day at which time they are completely replaced by embryonic tissue. The protrusions thus formed represent the so-called villi (Amoroso, 1952).

On about the ninth day, the trophoblast first becomes definitely attached to the endometrial capillaries and surrounds the endometrial arterioles. By the time the allantoic circulation is established, the endometrium in the subplacental region has become "transformed into closely packed perivascular sheaths, which can be spoken of in its entirety as decidua basalis" (Mossman, 1937).

The trophoblast, which has now destroyed the superficial and glandular epithelium, advances into the interglandular connective tissue, the cells of which degenerate and are absorbed (Mossman, 1926). These rapidly ingrowing processes of the trophoblast push in until they reach the endothelium of a capillary, then gradually work around its wall until they completely enclose the vessel which, although it retains its own endothelial wall, now lies in the midst of tissue of embryonic origin. Thus, the rabbit placenta temporarily exhibits an endothelio-chorial condition. However, by the tenth day the maternal capillaries which are completely surrounded by trophoblast have

entirely lost their endothelium and lie, as maternal spaces, in the midst of the trophoblastic lamellae. With this disappearance of the endothelium, the haemochorial condition is established (Amoroso, 1952). "The placenta formed in this way — by ingrowth of the trophoblast, is characterized by (1) the absence of foetal mesoderm, and (2) by the presence of degenerating maternal tissue and foetal syncytial lamellae enclosing spaces filled with maternal blood" (Mossman, 1937).

The mucous membrane, at this stage, is divided into two regions; the intermediary region (zone of ingrowth of Grosser) and the region of uterine sinuses (Mossman, 1926). Contact of invading trophoblast with the intermediary region results in "the decidual cells" increasing in size and becoming multinucleate. In the region of the uterine sinuses the blood vessels dilate forming large spaces and the decidual cells remain uninucleate. The junction between the two zones is marked by the blind ends of the glands which are filled with degenerating epithelium. In section each appears as an island of glandular symplasm (Mossman, 1926).

The placental trophoblast is now, at the tenth day, differentiated into two layers, the superficial syncytiotrophoblast and a deeper cellular layer, the cytotrophoblast (Mossman, 1937; Amoroso, 1952). The trophoblast is being invaded at intervals by processes of vascular mesoderm which break it into columns. At the same time, the embryonic tissues continue to advance and surround maternal capillaries. These vessels lose their original size and shape and become distorted into large irregularly shaped sinuses.

During this time, the allantois has grown out from the embryo into the

exocoel and has extended around the amnion. The outer wall of the allantois, made up of vascular splanchnic mesoderm, comes into contact and fuses with the inner surface of the serosa over a circular area on the mesometrial side where the trophoblast has invaded the maternal tissue. Thus the placenta is vascularized by the allantoic blood vessels (Mossman, 1926). Through the invasion of the placental trophoblast, the maternal blood in the placenta comes to circulate through a labyrinth of tubular channels or lacunae in the thickened plasmodial trophoblast. With the vascularizing of the trophoblast by allantoic mesoderm and blood vessels and the destruction of maternal endothelium, the only layers separating the two blood streams are embryonic endothelium, strands of mesenchyme, cellular trophoblast and thin syncytial trophoblast (Mossman, 1926; Brambell, 1952; Amoroso, 1952).

Finally, according to Mossman (1926), the capillaries project through the trophoblastic walls into the maternal blood in the lacunae. At these points, endothelium alone separates the maternal and embryonic blood (Mossman, 1926). According to Brambell (1952) this condition is attained by the end of the fifteenth day _post coitum_ and the placenta is fully established at this time. However, Amoroso (1952) contends that this condition is not reached until the twenty second day.

MATERIALS AND METHODS

Materials for this project were obtained from animals maintained in a laboratory for this work at Kansas State University.

Female New Zealand white rabbits, kept under optimal, isolated condi-

tions, were bred to two males as a protective measure against male sterility. Due to failure of the females to ovulate during the fall and winter, intravenous injections of from 20-35 I. U. of Upjohn's Gonadogen (PMS) were given to stimulate ovulation in some of the first animals used. During this time, forced breeding techniques were necessary due to failure of the female to stand for the male. However, during the course of this work, a time-switch was installed in the laboratory which maintained well lighted conditions in the room for 15 hours a day. The females thereafter remained in estrus continuously and failure to stand for copulation or to ovulate has not been observed at any time during the entire year.

The stages for this work were aged according to time post ovulation, day one beginning ten hours post coitum.

At the desired interval after ovulation, the animals were anesthetized with Nembutol, which was diluted one part Nembutol to two parts sterile saline. This dilution was found necessary because of the extreme difference of sensitivity of different rabbits to Nembutol. Because of this factor, a new anesthetic was desired. It was found that ethyl carbamate in the proportion of 0.10-0.12 grams per kilogram weight of rabbit administered rectally gave a much safer, more relaxed anesthesia than did Nembutol. It also gave the advantage of greater ease of administration.

Laparotomy was performed under aseptic conditions, the left uterine horn tied off just above the bifurcation and removed along with the corresponding ovary. The incision was then closed and the rabbit placed in a recovery cage. The animal was sacrificed 12 hours later and the right uterine

horn and its corresponding ovary removed.

Immediately after excision, the uterine horn was stretched in a wax-bottom tray and fixed with 10% buffered formalin for about one hour. The embryonic swellings were then separated, trimmed and placed in Bouin's fluid diluted 1:1 with 5% acetic acid. In some of the larger specimens the thin antimesometrial surface of the uterine swelling was opened after about one hour of fixation to allow the fixative to reach the placental region more effectively. In others, from 0.1 to 0.7 ml of Bouin's fluid, depending on the size of the swelling, was injected directly into the cavity of the uterine swelling through the antimesometrial wall.

The specimens were fixed 48 hours, dehydrated in isopropyl alcohol, embedded in paraffin and serially sectioned at 8 microns. Sections were stained with, (1) a modified Harris hematoxylin, counterstained with acid fuchsin-Orange G, or with, (2) Mallory's triple stain. Some slides from each series were stained with periodic acid-Shiff's (PAS) to show special relationships.

OBSERVATIONS

Nonpregnant Uterus

The rabbit uterus has the basic structure of that of a typical mammal, with certain specializations. It has an irregular lumen, a variable endometrium comprised of epithelium, uterine glands, stratum compactum and stratum spongiosum, and a myometrium consisting of circular and longitudinal muscle (Fig. 1, Fig. 2).

The endometrium of the mesometrial surface consists of two heavy ridges, the "mesoendometrial ridges", one on each side of a groove in the endometrium, the "mesoendometrial groove" which is in direct line with the mesometrium. These ridges are regularly zig-zagged along their entire course with a point of one extending in toward the mesoendometrial groove directly across from where a point on the other ridge runs away from the groove. This makes the mesoendometrial ridges interlock in much the same manner as a closed zipper (Fig. 24). The "periendometrial ridges" are nonconsistent, lying to each side and matching the course of the mesoendometrial ridges. Irregular "antimesoendometrial ridges" lie along the remainder of the uterine wall not covered by the mesoendometrial and periendometrial ridges. They vary in number from three to five and are low, flat, ribbon-like ridges which are convoluted both longitudinally and vertically in an irregular manner.

The epithelium of the endometrium is low to high columnar, 12 to 15 microns in thickness. Individual cells vary in height/diameter ratio from 4:1 to 6:1. The uterine glands are short, essentially straight pockets of epithelium, continuous with the uterine epithelium (Fig. 2). The glands of the mesoendometrial and periendometrial ridges are only slightly larger and deeper than are those in other parts of the uterus. The epithelial cells in the glands, forming the "glandular epithelium", are generally taller than those of the "luminal epithelium" with nuclei more basal.

The stratum compactum is a distinct layer of connective tissue, oriented parallel to the epithelium and filling in between the necks of the uterine glands. The stratum spongiosum is composed of dense, irregular, connective

tissue between the distal ends of the uterine glands and makes up the core of the endometrial ridges (Fig. 2).

The blood vessels of the stratum compactum consist of small, thin-walled capillaries, dispersed sparsely. The stratum spongiosum is, on the other hand, well vascularized; capillaries are extremely numerous with as many as 12 showing in a section 0.1 x 0.1 mm. Larger arteries and veins are found here than in the stratum compactum. The mesoendometrial ridges are somewhat more heavily supplied with blood vessels than are other parts of the endometrium.

The myometrium is composed of an outer layer of longitudinal muscle approximately 0.7 mm thick and an inner layer of circular muscle approximately 0.6 mm thick. Both layers are penetrated by connective tissue bundles associated with the blood vessels (Fig. 1).

Six Day Post Ovulation

By the beginning of the sixth day of gestation the trophocysts have reached their definitive positions in the uterus and have expanded, stretching the antimesometrial surface until definite swellings are visible grossly.

The myometrium in the antimesometrial surface is slightly thinner than in the nonpregnant condition but that lying beneath the mesoendometrial ridges remains unchanged. The endometrium across the antimesometrial surface is reduced, mainly by stretching of stratum spongiosum. The irregular endometrial ridges flatten out as the uterus swells with the expanding

trophocyst. The mesoendometrial ridges enlarge by increase of the stratum spongiosum and uterine glands, protruding deeply into the lumen and become the dominant structure in the uterus (Fig. 3). The uterine epithelium remains essentially unchanged over the entire surface. In the uterine glands, however, numerous mitotic divisions occur as the glands penetrate more deeply into the expanding mesoendometrial ridges. The epithelia of both the luminal surface and the glands consist of simple columnar cells, changing to cuboidal in the deep glands.

Although the trophocyst has reached its final position in the uterus and has stretched the uterus at this point, there is, as yet, no actual contact between trophoderm and uterine epithelium because the intact zona pellucida is still tightly adhered to the trophocyst. The trophoderm has begun to form a few specialized structures, the "trophoblastic nodules", consisting of syncytial spheres with the nuclei located around the outside, leaving the center a cytoplasmic mass (Fig. 4). These trophoblastic nodules vary from 40 to 70 microns, involve the entire thickness of the trophoderm, are completely limited externally by zona pellucida, and occur at irregular intervals without reference to endometrial ridges or maternal blood vessels.

Summary. By the sixth day of gestation the trophocysts, with the zona pellucida still intact, have reached their definitive positions in the uterus. Across the antimesometrial surface the myometrium and stratum spongiosum are slightly thinned and the antimesoendometrial ridges are being flattened by the expanding trophocyst. The mesoendometrial folds have expanded by an increase in the amount of stratum spongiosum present and depth of the

uterine glands. The uterine epithelium remains essentially unchanged. Trophoblastic nodules have begun to form on the trophoderm.

Six and One-Half Day Post Ovulation

At this stage of pregnancy, the myometrium has been stretched, thus reducing the thickness in that part of the uterus in which the embryo is located. The longitudinal muscle is now 0.4 to 0.5 mm thick and the circular muscle has been reduced to 0.3 mm.

The uterine epithelium has undergone great expansion in both the glands and on the surface. The cells of the luminal epithelium have become much more compact to the extent of changing to a pseudocolumnar condition with two or three layers of nuclei (Fig. 5). The uterine glands have become elongated, branched and somewhat coiled. The epithelium in the glands varies from columnar in the neck to cuboidal at the tip, indicating growth terminally (Fig. 5). There is no line of demarcation between luminal and glandular epithelium other than a transition from pseudocolumnar to columnar in the neck of the glands.

On the antimesometrial surface, the endometrial ridges have been stretched by the expansion of this surface in accomodating the growing trophocyst. The uterine epithelium has undergone extreme proliferation concurrent with the expansion of the lumen. The stratum compactum has lost its distinctness and the stratum spongiosum remains only as a thin band of vascularized connective tissue, about 0.1 mm thick, between the glands. The mesoendometrial ridges have expanded until they are about 1 mm high and 1.5 mm

across, with a core of stratum spongiosum into which the expanding uterine glands penetrate. Only a thin layer of stratum compactum remains between the necks of the glands. The uterine glands in these ridges are elongated to as much as 0.7 mm; much more than are those of the antimesoendometrium. Blood vessels in the mesoendometrial ridges have become greatly expanded and have lost most of their muscle and adventitia, permitting leakage of fluids into the surrounding tissue. This leakage is a great factor in the expansion of the stratum spongiosum, as this expansion has come about by an increase in intercellular space rather than an increase in the number of cells.

The trophocyst, at this stage, is a bilaminar omphalopleur and has become oriented in the uterus with the embryonic pole directly above the mesoendometrial groove. The zona pellucida still encloses the trophoderm (Fig. 6), which is composed of a single layer of cuboidal cells at the embryonic pole which thin to squamous cells on the abembryonic hemisphere. The inner layer of the embryonic vesicle is a single layer of entodermal cells which take a much lighter stain than do those of the trophoderm.

Summary. At six and one-half days, the zona pellucida still encloses the trophocyst and no trophoderm-uterine epithelium contact has been made as yet. The myometrium is reduced, especially on the antimesometrial surface as is the stratum spongiosum of this area. The mesoendometrial ridges have increased in size by expansion of the stratum spongiosum and growth of the uterine glands. The uterine epithelium is still composed of distinct cells which have proliferated on the luminal surface and in the glands until they are compacted into a pseudocolumnar condition in places.

Seven Day Post Ovulation

At the beginning of the seventh day of gestation, the greater part of the endometrium remains unchanged. The luminal epithelium on the mesometrial surface has proliferated to an even greater extent until it now has two to four layers of nuclei. The epithelium in the glands again varies from a tall columnar near the neck to a proliferating cuboidal near the base. The epithelium in the necks of the glands is transitional between the pseudocolumnar surface and the simple columnar of the glands. The cells of the epithelium have begun to produce large amounts of a granular, PAS positive material, retained within the cell, greatly distending the cell membrane in an irregular manner. The production of this material is much greater in the glands than on the surface, expanding the gland cells into the lumina of the glands, almost filling them in places (Fig. 7).

The uterine glands on the antimesometrial surface have increased in length until they approach the myometrium and curve out along it. The necks of the glands are progressively converted into luminal epithelium by the stretching of the wall of the uterus, thus reducing the length of the gland from the luminal end while the tip is elongating by proliferation. On the mesoendometrial ridges the glands are elongated and coiled, reaching to within 0.2 mm of the myometrium.

The ridges of the antimesometrial surface have been almost entirely flattened by the stretching of the uterus and the only connective tissue remaining is that carrying the blood vessels between the glands. The meso-

endometrial ridges have increased until they are about 1.5 mm tall and up to 2 mm across the base. The stratum compactum of these ridges has now lost its distinctness between the necks of the uterine glands. The stratum spongiosum is responsible for much of the increased growth of the ridges and again shows a much greater increase in intercellular space than in the number of cells. The blood vessels are more expanded and all endometrial vessels now consist of endothelium with little or no muscle layer.

The zona pellucida has broken, usually in many places, as if it were being differentially dissolved, allowing direct contact between trophoderm and endometrium. The remaining zona undergoes a curling action which draws it into a much smaller area. In all embryos of this stage studied, there were yet patches of zona between trophoderm and endometrium (Fig. 8).

In the embryonic hemisphere, the trophoderm is two to four layers of nuclei thick, and a thin layer of mesoderm is forming between trophoderm and endoderm. No fusion of any kind with the uterine epithelium has taken place over this surface (Fig. 9).

All the embryonic vesicle except that directly adjacent to the meso-endometrial ridges consists of a bilaminar omphalopleur, with single cell layers of endoderm and trophoderm except for the trophoblastic nodules. Part of the trophoblastic nodules have made contact and fused with the uterine epithelium, while zona pellucida yet covers some of the nodules, particularly over the periendometrial ridges.

As the trophoblastic nodules connect with the epithelium, the fused cell membranes dissolve and the contents of nodule and epithelium "flow"

together, forming a symplasm comprised of both embryonic and maternal material. In some of the most recent sites of attachment, both embryonic and epithelial nuclei may be seen mixed together (Fig. 10), but in older attachments the nuclei of the epithelium has disappeared. The uterine epithelial cells other than those in the attachment areas are normal and distinct. In no case was the invasion by the trophoderm observed to go beyond the epithelium into the connective tissue, nor the symplasmic condition to extend beyond the limits of fusion (Fig. 10). Except for these antimesometrial attachment sites, both the luminal epithelium and the glandular epithelium over the entire surface are distinctly cellular, with no indication of a syncytium or symplasm.

Summary. At seven days post ovulation, the zona pellucida has been partially shed and contact has begun between trophoderm and uterine epithelium. The only fusion is over the antimesometrial surface and there only where a trophoblastic nodule has made contact with uterine epithelium. The antimesoendometrial ridges have been flattened until this surface is virtually smooth. The mesoendometrial ridges continue to expand. The cells of the uterine epithelium remain as distinct, columnar to pseudocolumnar cells, except where trophoblastic nodules have invaded. The cells of the glandular epithelium have begun to produce a granular, PAS positive intracellular secretion.

Seven and One-Half Day Post Ovulation

The uterine epithelium has reached its greatest proliferation at this stage. The cells on the surface between the necks of the glands are so packed together that the nuclei appear as a jumbled layer and it is, in a few places, two cells thick. This greatly thickened condition is most extreme on the mesoendometrial ridges. Such "crowding" of cells has not occurred in the uterine glands; after an area of transitional epithelium in the necks of the glands, the glandular epithelium tapers off to its normal tall columnar to cuboidal type. All the cells of the epithelium of the mesoendometrial ridges, especially those in the glands, remain active in the production of the droplets which began in the seven-day stage (Fig. 7). In many of the glands, the cells have expanded to such an extent that no lumen remains.

The uterine glands along the antimesometrial surface have been stretched out straight except for the basal portions which lie along the myometrium. Connective tissue in this area is almost nonexistent except for the adventitia of the blood vessels and tiny, compact strands connected to these vessels. The myometrium over this hemisphere is reduced to a thickness of no more than 0.3 mm, both layers being approximately equal.

On the mesoendometrial ridges, the uterine glands have become even more elongated and coiled. Some reach the entire distance to the myometrium and all are woven through the stratum spongiosum in such a manner as to make it impossible to follow any one gland very far in one section. The stratum compactum is nonexistent in places and is an extremely thin layer where it

persists. The blood vessels of these ridges have increased in number, especially in the areas near the uterine epithelium. These vessels consist of an endothelial lining with little if any muscle or adventitia and are expanded beyond the size of a normal capillary.

The trophocyst has enlarged, further expanding the uterine swelling. Mesoderm has become more widespread along the embryonic pole of the trophocyst but shows no sign of splitting into somatic and splanchnic layers. The trophoderm in the abembryonic hemisphere still consists of a single layer except for the enlarging trophoblastic nodules, most of which are attached to the uterine epithelium by this time. The syncytium of the trophoblastic nodules has spread out along the surface of the endometrium, destroying and replacing the epithelium as it goes. No invasion of subepithelial tissue was found. The trophoderm in the embryonic hemisphere has undergone extreme thickening until the portion directly over the mesoendometrial ridges is now 5 to 8 nuclei thick, tapering off over the periendometrial ridges to the typical single cell layer. The central portion of this thickened trophodermal plate is distinctly differentiated into two layers: a "cytotrophoderm" next to the blastocoel and a "syncytiotrophoderm" next to the mesoendometrial ridge (Fig. 11). The syncytiotrophoderm is a plate of trophoblast, 25 to 30 microns thick in the middle, tapering to 2 to 3 microns at the edges. Nuclei are contained within a lightly staining cytoplasm that shows no cell membrane except on the surface with any combination of fixatives and stains that have been tried. The nuclei of this syncytium form a compact layer next to the cytotrophoderm with a layer of clear cytoplasm toward the epithelium. There

is as yet no fusion between serosa and uterine epithelium in this area, both membranes remaining entirely intact and separate as they were in the seven day stage (Fig. 9).

Summary. The zona pellucida has disappeared by this stage and the number of attachment points with the antimesoendometrium has increased but no invasion of the endometrial connective tissue has occurred. The trophoderm of the embryonic hemisphere is now composed of two distinct layers, the cytotrophoderm and the syncytiotrophoderm. There has been an increase in the number and size of the blood vessels in the mesoendometrial ridges. The uterine epithelium is greatly thickened.

Eight Day Post Ovulation

On the antimesometrial surface, from the edge of each periendometrial ridge to the edge of the other, the epithelium and the entire endometrium have undergone extreme degeneration until they appear as a dead, decaying symplastic mass. This degeneration occurs not only in the endometrium but also in the trophoderm and trophoblastic nodules. It is violent and sudden and appears to be the result of a very highly destructive agent, perhaps produced by the trophoblastic nodules. The only intact cells remaining of the original endometrium are epithelial cells in a partial layer from some of the deeper parts of the original uterine glands next to the myometrium and thin strands of connective tissue between these (Fig. 12).

On the portions of the mesoendometrial ridges lateral to the embryo, the syncytiotrophoderm has fused with and is destroying the uterine epithelium

and underlying connective tissue. There is no fusion in the area directly underlying the embryo. On the outer edges of the attachment area, the cell membranes of the uterine epithelium have fused with the membrane of the syncytiotrophoderm. In the portions of the mesoendometrial ridges where attachment occurred first, this fusion membrane has broken down along with the cell membranes between the epithelial cells, forming a symplasm containing nuclei of both syncytiotrophoderm and epithelium. However, the nuclei of the epithelium are quickly destroyed once the cell membranes disappear. The destruction is much like that which occurred in the attachments on the antimesometrial surface but does not develope into the extreme destruction demonstrated in that area. This may be due to the destructive agent produced in the syncytiotrophoderm being weaker than that of the trophoblastic nodules, or the extensive blood supply of the mesoendometrial ridges being able to neutralize the agent more rapidly. The endometrium is penetrated at regular intervals by pseudopodia-like projections from the syncytiotrophoderm, made up of bundles of nuclei behind an advancing front of cytoplasm (Fig. 13). Some of these projections extend into necks of uterine glands while others penetrate directly into solid endometrium. The projections seldom extend any further into a uterine gland than the line of destruction of the surrounding endometrium. The cell membranes may be seen degenerating immediately ahead of the advance of the invading trophoderm. The deepest penetration at this stage is not over 0.18 mm.

The only uterine epithelium remaining intact is that on the inner edges of the periendometrial ridges, on the parts of the mesoendometrial ridges

which have not been invaded by trophoderm, and in the deeper portions of the uterine glands. The remaining portions of uterine glands are still elongate and coiled and have changed little from the previous stage. The stratum compactum has become less and less tightly packed until it now cannot be distinguished from the stratum spongiosum and therefore no longer exists as a separate part of the endometrium. The mesoendometrial ridges themselves have not increased measurably in volume.

The trophocyst has undergone extreme modification at this stage. The trophoderm has been destroyed along the antimesometrial surface (Fig. 12), leaving yet intact the single-celled layer of endoderm, separated from the living uterine wall by the debris of the destroyed endometrium and trophoderm. The embryo proper is in the early neural tube stage. The amnion has covered the head. No allantois is visible at this stage. The mesoderm has filled the blastocoel over the entire mesoendometrial ridges, split into splanchnic and somatic layers and the splanchnic layer has developed blood islands and distinct vitelline vessels containing nucleated blood cells. The two layers of mesoderm lie in direct contact with each other so that the exocoel is, at most, a very narrow slit. There is no sinus terminalis. In the embryonic hemisphere, the parts of the trophoderm not attached to the endometrium, remain distinctly divisably into cytotrophoderm and syncytiotrophoderm, the nuclei of the latter remaining in a thick layer next to the cytotrophoderm. The thickeness of the entire layer is variable in the amount of cytoplasm between the nuclei and the uterine epithelium.

Summary. The endometrium and trophoderm over the antimesometrial

surface have degenerated and remain only as a dead mass between the endoderm of the trophocyst and the few intact epithelial cells remaining from the deep uterine glands. The syncytiotrophoderm has invaded and destroyed the epithelium of the mesoendometrial ridges laterally to the embryo and is penetrating the underlying stratum spongiosum by means of cytoplasmic projections. In the few places where invasion has not taken place, the cells of the uterine epithelium remain intact and in a normal condition.

Nine Day Post Ovulation

At the beginning of the ninth day of gestation, little of the original uterine epithelium remains around the embryonic vesicle. Only on parts of the periendometrial ridges and mesometrial groove is the epithelium intact and both luminal and glandular cells remain unchanged.

On the antimesometrial surface, the epithelium and uterine glands, along with most of the stratum spongiosum, the trophoderm, and the trophoblastic nodules have degenerated further and remain only as symplastic masses of pycnotic nuclei and lifeless cytoplasm between the remnant of endometrium and the embryonic endodermal vesicle. This condition of degeneration and sloughing of endometrium includes as much as a third of the periendometrial ridges. The portions of the uterine glands, which lie along the myometrium and were not reached by the destructive agent, have begun to proliferate rapidly by the beginning of the ninth day and normal cells spread over the surface forming the beginnings of a new uterine epithelium (Fig. 14). This new epithelium is incomplete so that in many places the uterine lumen is

void of an epithelial lining.

After the destruction of the trophoderm, the abembryonic hemisphere of the embryonic vesicle consists of a single layer of squamous endodermal cells (Fig. 14).

The embryo at nine days post ovulation has a closed neural tube and the amnion is complete over its entire length. It lies directly above the meso-endometrial groove. The exocoel is large, with the somatic mesoderm in direct contact with the cytotrophoderm and the highly vascularized splanchnic or yolk sac mesoderm in contact with the endoderm as far as the sinus terminalis. There is now a definite sinus terminalis as a large blood sinus directly over the outer margins of the mesoendometrial ridges. The allantoic diverticutum is present but does not as yet extend beyond the lateral body folds although the body stalk is a broad mesodermal connection between embryo and serosa and is penetrated by "allantoic" blood vessels containing embryonic blood cells.

The uterine epithelium and uterine glands of the mesoendometrial ridges remain unmodified only deep in the mesoendometrial groove. A few epithelial cells remain at the tips of some of the deepest uterine glands but within most of the ridges, these have already begun to degenerate in front of the advancing syncytiotrophoderm.

The advancing front of cytoplasm of the syncytiotrophoderm is divided into pseudopodia-like "plasmodia" which press into the endometrium in a random manner, destroying, digesting, and engulfing maternal tissue as they go. These projections may surround an area of endometrial tissue and proceed deeper into the endometrium while the destruction of the surrounded tissue is

still taking place or they may destroy and absorb the maternal tissue directly ahead of their advance. The cells of the endometrium directly ahead of the advancing plasmodia are in a state of early degeneration as shown by irregular cell membranes and pycnotic nuclei. The blood vessels are much more slowly destroyed and are therefore bypassed and surrounded by the plasmodia. As the blood vessels are progressively destroyed, the maternal blood flows through channels in the syncytiotrophoderm (Fig. 15). The previous heavy masses of nuclei of the syncytiotrophoderm have now become dispersed behind the cytoplasmic plasmodia. These nuclei are in clumps of from two to 12, each clump becoming enclosed by a cell membrane thus forming multinucleate cells from the original syncytium. These cells fill in the exact area previously occupied by the endometrium and form the structure of the "placental pad".

Following behind the destructive invasion of the syncytiotrophoderm, the cytotrophoderm produces straight, tubular villus projections 0.1 to 0.25 mm in length and 0.1 to 0.2 mm in diameter (Fig. 16), which penetrate into the placental pad by active proliferation of the cells in the tips of the villi. These villi grow into and replace the syncytiotrophoderm except for a layer around each blood channel. In extreme conditions at this stage, the villi completely surround the blood channels until the vessel has a wall made of cytotrophoderm with an endothelial-like lining of syncytiotrophoderm. Each cytotrophodermal villus has a definite core of serosal mesoderm (Fig. 16). The embryonic blood vessels from the body stalk have begun to spread into

some of these mesodermal cores of villi near the body stalk.

Summary. By the ninth day post ovulation, the epithelial cells of the uterine glands which remained intact on the antimesometrial surface have begun to proliferate, expanding over part of the bare surface left by the destruction of endometrium in this area. The endoderm of the bilaminar omphalopleur was not destroyed in this reaction and remains intact. On the mesometrial surface, the luminal epithelium is gone and plasmodia of the syncytiotrophoderm extend into uterine glands and stratum spongiosum, digesting maternal tissue in their path. As these plasmodia surround maternal blood vessels, the walls of the vessels are destroyed and the blood flows through channels in embryonic tissue. Behind the syncytiotrophoderm, the cytotrophoderm has begun to produce villi which project into the syncytiotrophoderm, destroying this tissue as it goes.

Ten Day Post Ovulation

Over the antimesometrial surface, the new uterine epithelium being regenerated from the remnants of the uterine glands has progressed rapidly so that only a few areas remain without an epithelial covering. The beginnings of a new stratum spongiosum are also under way as proliferation of the remnants of the original spongiosum increases.

The endoderm remains as the only layer of the abembryonic hemisphere of the embryonic vesicle. The embryo itself has increased considerably in size from the previous stage. The amnion is complete and there is as yet no sign of the allantoic diverticulum extending beyond the body cavity

although allantoic blood vessels extend deeply into the villous cores of the placenta. The sinus terminalis is distinct and the lateral mesoderm extends slightly beyond it, joining yolksac endoderm and trophoderm.

Destruction of the periendometrial ridges has progressed variably. In some specimens, only the surface layer has been destroyed; in others, nearly the entire ridge has degenerated with only tips of uterine glands and associated tissues remaining. The mesoendometrial ridges, now mostly placental pads (Fig. 17), have enlarged by addition of embryonic tissue in excess of that which has replaced maternal tissue. The destructive advance of the syncytiotrophoderm (Fig. 17) reaches to within a few microns of the myometrium. The pseudopodial projections of the syncytiotrophoderm have destroyed everything in their paths, including the muscles and endothelium of the maternal blood vessels. The entire path of each blood vessel had been completely surrounded by the syncytiotrophoderm before the maternal endothelium was destroyed so that maternal blood now flows through a channel in embryonic tissue which maintains the original path of the maternal vessel.

The cytotrophodermal villi (Fig. 17) have advanced approximately half the distance from the surface of the placental pad to the limit of syncytiotrophodermal invasion. The tubular villi project almost straight into the pad and replace the syncytiotrophoderm by destruction and absorption as they go. The blood channels which were formed by syncytiotrophoderm along the path of the original maternal vessel are, in turn, surrounded and replaced by the cytotrophoderm (Fig. 18). Therefore, maternal blood, in the portion of the pad made up of cytotrophoderm, flows through a third type of channel, the

first being the original uterine vessel which was destroyed and replaced by the syncytiotrophoderm, which in turn was destroyed and replaced by the cytotrophoderm. The destruction and replacement of the different layers is accomplished in such a way that there is no leakage of blood into the tissue and by no means any formation of blood pools. These changes in the structure of the vessels are accomplished with no apparent change in the course of the vessel.

The mesodermal cores of the cytotrophodermal villi have become highly vascularized by allantoic blood vessels (Fig. 19) which extend from the body stalk. These embryonic vessels are thus in close proximity with the cytotrophodermal channels through which the maternal blood flows.

Summary. By the tenth day *post ovulation*, the new uterine epithelium of the antimesometrial surface is greatly expanded but areas remain without epithelial covering. Beginnings of a new stratum spongiosum are evident. The abembryonic hemisphere of the embryonic vesicle consists of a single layer of endoderm. The syncytiotrophoderm has destroyed endometrium to within a few microns of the myometrium and has itself been destroyed from above by the cytotrophoderm through almost half the depth of the placental pad. The villi of the cytotrophoderm are lined with highly vascular embryonic mesoderm. The villi have surrounded the vessels of syncytiotrophoderm which carry maternal blood and have, in part, replaced the syncytiotrophoderm, leaving the maternal blood now flowing from the maternal capillaries below the base of the placenta into channels in syncytiotrophoderm in the basal portion of the placental pad into channels in cytotrophoderm where it has

replaced syncytiotrophoderm in the portion of the placenta nearer the embryo.

Eleven Day Post Ovulation

By the eleventh day of gestation, the only remaining areas of the original uterine epithelium are located in the deepest portions of the mesoendometrial groove. The periendometrial ridges are almost gone, the epithelium and most of the stratum spongiosum having been destroyed as were those of the antimesometrial surface. Regeneration of the new uterine epithelium is essentially complete and the stratum spongiosum beneath it has become highly vascularized.

On the antimesometrial surface, the trophoderm and the original uterine epithelium remain as only scattered debris between the regenerating epithelium and the endoderm, which is still essentially intact and constitutes the entire abembryonic hemisphere of the embryonic vesicle. The new endometrium being regenerated shows no development of endometrial ridges. There are a few, shallow uterine glands scattered at random but the entire thickness of the endometrium does not exceed 0.4 mm in this area. Giant cells comprise a conspicuous part of the endometrium at this time. The embryo is approximately 5 mm in crown-rump length. The sinus terminalis is no longer at the outer edge of the exocoel since the mesoderm extends beyond it a considerable distance. The exocoel is increasing rapidly and the fused mesoderm and endoderm of the yolk sac is being pushed toward the single layer of endoderm covering the abembryonic hemisphere of the embryonic vesicle. The allantoic diverticulum still does not extend beyond the embryonic coelom

proper.

Destruction of the endometrium is essentially complete with invasion of the syncytiotrophoderm having extended deeply into the mesoendometrial ridges, destroying all of the uterine glands in the region of the placental pads. The portion of the stratum spongiosum remaining below the margin of invasion has become extremely vascularized with maternal capillaries. Along the base of the placental pad, clumps of nuclei of the syncytiotrophoderm and the invading plasmodia have been compressed into a thin layer, forming a distinct line of demarcation between maternal tissue and the vascularized embryonic part of the placenta. The placental pad is a near-circular disc, thin on the lateral edges and deepest along the mesoendometrial groove.

The villi of the cytotrophoderm have penetrated deeply into the pad, almost overtaking the advanced front of the syncytiotrophoderm. The cytotrophodermal villi have surrounded maternal blood channels in their path by adjoining villi separating around a channel, then coming together again as the tips of the villi extend beyond the channel. The layer of syncytiotrophoderm which originally surrounded and destroyed the endothelium of the vessel has in turn been destroyed and replaced by the cytotrophoderm, leaving now syncytiotrophoderm only around the blood passages that have most recently been engulfed. The mesodermal core of the cytotrophodermal villi is rich in allantoic blood vessels which consist of a single endothalial layer and extend the entire length of the villus. Where these vessels come in contact with the cytotrophoderm which surrounds maternal blood, the wall of the cytotrophodermal channel is thinned until embryonic blood vessels are usually

separated from maternal blood by only two or three cell layers. In a few places the cytotrophoderm appears to have been thinned to a single cell layer. The thinning of the cytotrophoderm must be quite rapid as the above described condition occurs from the base to the tip of the villus. There may be as many as five to eight allantoic blood vessels parallel to and in contact with one maternal blood channel.

Summary. By 11 days *post ovulation*, the uterine epithelium of the antimesometrial surface is essentially complete. The original abembryonic endodermal sac is still intact. The syncytiotrophoderm has destroyed and replaced all but a thin layer of the mesoendometrium. The cytotrophodermal villi have, in turn, destroyed and replaced the syncytiotrophoderm to within a short distance of its deepest penetration, making the placental pad composed mainly of cytotrophoderm. These villi remain lined with vascularized mesoderm, therby carrying embryonic blood vessels to the depths of the placental pad. Thinning of the wall of the villus where it surrounds a maternal blood channel has brought the embryonic blood into close proximity with maternal blood.

Twelve Day Post Ovulation

By 12 days, the antimesometrial surface of the uterine swelling is completely lined by the new uterine epithelium, derived from the deepest portions of the original uterine glands (Fig. 20). This new epithelium is a high columnar to pseudostratified columnar type and is smooth, with neither ridges nor uterine glands. Beneath the new epithelium, the stratum

spongiosum has begun to proliferate and is, in most cases, a continuous layer between myometrium and epithelium. A distinct characteristic of this layer is the giant cells which are abundant and form a complete layer in places (Fig. 21). They are large cells (50-100u) and nuclei vary from a single to as many as five. The cytoplasm is nongranular and the entire cell is oblong with the nucleus or nuclei usually at one end. These cells are most abundant in the stratum spongiosum of the antimesometrial surface but may be seen as deep as the longitudinal layer of the myometrium. They appear to be symplasmic masses of the original uterine epithelium which were injured but not completely destroyed by the trophoderm.

The embryo proper has increased in size and is connected to the placenta by a body stalk. The allantois has extended through the body stalk to the extent of touching the placental pad. At best it can be considered a rudimentary organ although its blood vessels are responsible for the vascularization of the placenta. The endodermal layer which lined the abembryonic trophoderm has begun to degenerate (Fig. 20) and the hemisphere of the yolk sac nearest the embryo remains intact.

In the area of the mesoendometrium, the stratum spongiosum below the extent of trophoblastic invasion has proliferated and formed a heavy layer between myometrium and placenta. This tends to push the pad out into the lumen of the uterine swelling. The placental pads have increased greatly by proliferation of the cytotrophoderm in excess of the mass needed to replace destroyed maternal tissue. This increase in size causes an overgrowth of the free lateral trophoderm by the placental pad and makes it appear as if the

trophoderm were undercutting the placenta. The cytotrophoderm has completely overtaken the syncytiotrophoderm and only a few remnants of the latter may be found scattered along the base of the placental pad. The villi of cytotrophoderm reach the entire distance to the base of the pad, carrying allantoic blood vessels in their mesodermal cores thus extending the close association of allantoic vessels and maternal blood channels considerably beyond the condition noted at 11 days. The major change in this stage is the great expansion of maternal blood channels and allantoic blood vessels along the surface of the placental pad. Expanded sinuses are formed in the surface vessels of the pad where there is lack of support of surrounding tissues which the deeper vessels have and the noncollagenous cytotrophodermal walls will not hold up under the maternal blood pressure. The passages balloon into oversized channels but do not rupture in normal cases. The allantoic blood vessels which lie along the outer surface of these large channels are also oversized probably also from lack of support on the surface and supporting fibers in their walls.

<u>Summary</u>. By 12 days <u>post ovulation</u>, the antimesometrial surface is covered by a complete, smooth uterine epithelium which was regenerated from the portions of the uterine glands which survived the destruction of this area. The giant cells are very abundant in the new stratum spongiosum and the myometrium of this area. The cytotrophoderm has replaced the syncytiotrophoderm and is proliferating, thusly increasing the size of the placental pads. Below the placental pads, the stratum spongiosum which was not destroyed has begun to proliferate and form a dense layer which is heavily

vascularized. Both maternal and embryonic blood channels have greatly expanded along the surface of the placental pad.

Fifteen Day Post Ovulation

The antimesometrial surface of the uterine swelling has expanded greatly from the twelfth to the fifteenth day. The uterine epithelium is comprised of a single layer of cuboidal to columnar cells. The surface is essentially smooth and flat except for a small number of pits which appear to be the beginnings of new uterine glands. The stratum spongiosum has regenerated variably so that it is as much as 0.25 mm thick in some places while consisting of only a few thin strands in others. The giant cells are still a conspicuous part of the antimesometrium, occurring in great numbers in the new stratum spongiosum as well as in both layers of the myometrium. These often number up to 15 in a one mm portion of this region. These cells have begun to show early signs of degeneration at 15 days in that both their cell and nuclear membranes have become irregular and indistinct and in places where several are grouped together the intercellular membranes have broken and large symplasms are formed. Maternal blood channels are numerous and irregularly scattered throughout the new stratum spongiosum and are especially large and numerous in the myometrium. In the blood vessels of both regions which are directly adjacent to giant cells there are tremendous numbers of leukocytes, in some cases filling the entire lumen of the vessel. Leukocytes also occur in great numbers in the surrounding tissues. There is still a large amount of debris in the uterine lumen from the degeneration of the

original endometrium and trophoderm.

By 15 days the embryonic endoderm which remained after the degeneration of the trophoderm has degenerated thus completing the destruction of the original abembryonic hemisphere of the embryonic vesicle, leaving the endoderm of the yolk sac exposed to the uterine cavity. The remaining yolk sac has been expanded by increase of amniotic cavity and exocoel, filling the uterine lumen. It consists of two cell layers. The outer layer is composed of cuboidal to short columnar yolk sac endoderm cells which contain large, vacuoles filling most of almost every cell. The inner lining is of squamous mesodermal cells, highly vascularized in portions directly lateral to the embryo but remaining nonvascular beyond the sinus terminalis where it reflexes as serosal mesoderm. The allantois has, by 15 days, extended out of the body cavity through the body stalk and expanded to some extent over the surface of the placental pad. The allantois is a sac composed of a layer of cuboidal endoderm expanding within the mesoderm of the body stalk, between the vessels which are responsible for the vascularization of the placenta.

The placenta has expanded by proliferation of cytotrophoderm and protrudes far into the lumen of the uterus. The stratum spongiosum which had been reduced to a thin layer by the invasion of the trophoderm at 12 days has now regenerated until it is a thick, highly vascularized layer of up to one mm directly beneath the placenta, tapering off laterally to the thin, variable layer of the antimesoendometrium. The mesoendometrial groove is completely obliterated by embryonic tissue. The placenta is now a solid, discoid structure located directly over the site of attachment of the mesometrium.

<u>Summary</u>. During the twelfth through the fourteenth days of gestation the antimesoendometrial epithelium is complete and early development of uterine glands has begun. The giant cells are much in evidence but show signs of degeneration and have become surrounded by leukocytes. The endoderm of the bilaminar omphalopleur has broken down and the remaining yolk sac endoderm faces the uterine lumen. The allantois has extended through the body stalk. The stratum spongiosum underlying the placenta and the placenta itself have expanded until the placenta protrudes deep into the uterine swelling.

Twenty Day Post Ovulation

By the twentieth day of gestation, the placenta of the rabbit is morphologically complete. The antimesometrial surface has been expanded greatly in size by the enlarging amniotic vesicle, the abembryonic hemisphere of which is now covered by yolk sac splanchnopleur. The uterine epithelium of the antimesoendometrium is made up of tall columnar cells and displays short, straight uterine glands but no ridges are evident. The stratum spongiosum of this surface is a constant, complete layer of connective tissue between the epithelium and the myometrium. The giant cells are still evident but are reduced in number from the 15 day stage. Those remaining giant cells show signs of degeneration in that the nuclei display irregular nuclear membranes and heavy granules and the plasma membrane is irregular and appears to have broken in some cases. The concentration of leukocytes, both in the vessels and in the tissue around the giant cells is still at a very high level.

The placental pad continues to expand by proliferation of the cytotrophoderm. The villi have become indistinct as all the syncytiotrophoderm between them is destroyed and replaced. The embryonic blood vessels which were carried deep into the placenta in the mesodermal cores of the villi, permeate the tissue of the pad in all directions. Where an embryonic vessel contacts a maternal blood channel, the cytotrophoderm surrounding this channel has thinned to varying degrees (Fig. 22, Fig. 23). This includes cases in which the cytotrophoderm consists of as much as two cell layers, leaving three cell layers separating embryonic and maternal blood, the third being the endothelium of the embryonic blood vessel, to the extreme case where the cytotrophoderm has completely disappeared, leaving the embryonic capillary apparently extending into the maternal blood channel with only the single layer of embryonic endothelium separating embryonic and maternal blood.

Summary. The placenta of the rabbit is complete by 20 days gestation with a complete antimesoendometrium which displays rudimentary uterine glands and degenerating giant cells. The placental pad is composed of cytotrophoderm which is permeated with embryonic blood vessels and channels through which maternal blood flows. The embryonic and maternal blood is separated by varying thicknesses of tissue from three to one cell layer.

DISCUSSION

Although the general outline of mammalian placentation is followed in the rabbit, there are many obvious specializations which are peculiar to the rabbit and must be clearly defined and understood in order to study the

function of the placenta.

Most authors have chosen to age embryos or placentae in terms of time post coitum. However, since it has been well established (Walton and Hammond, 1928) that ovulation occurs ten hours after copulation with only slight variation, stages of development can be much more accurately timed in terms of days post ovulation with day one beginning ten hours after copulation. This gives a much clearer picture of the rate of advancing stages by eliminating the lag of almost one-half day when embryonic development is limited to progressive stages of capacitation of sperm and maturation of ovum.

In the nonpregnant rabbit uterus, there are two longitudinal, undulating ridges, the mesoendometrial ridges, which are located on each side of the mesoendometrial groove. These correspond to the "placental folds" of Hollard (1863) and Minot (1890) but the term ridge is more fitting since there is no folding in of the external surface of the uterus or even the endometrium to correspond with the growth into the lumen of the mesoendometrial ridges. Although in tracing through serial sections of a rabbit uterus, it might appear that the mesoendometrial ridges are divided by transverse fissures into rectangular areas, the "cousinets" of Hollard (1863), it can be seen by gross examination of an opened uterus that these ridges are solid, though undulated, structures and only the undulations make them appear in sections to be divided into knobs. The remainder of the endometrium is ridged to some extent with the periendometrial ridges, which lie to each side of the mesoendometrial ridges and correspond to the peri-placental folds of Minot (1890), and the antimesoendometrial ridges, which are irregular ridges that cover the

antimesometrial surface of the uterine lumen and correspond to the ob-placental folds of Minot (1890).

The trophocysts of the rabbit are spaced and oriented in the uterine lumen by six and one-half days post ovulation, which agrees with the seven day post coitum stage reported by Boving (1952) and Mossman (1926). The zona pellucida is still intact and no maternal-embryonic attachment has been made. Amoroso (1952), Assheton (1895), Brambell (1951), and Boving (1954), all reported the zona pellucida to be shed early in the eighth day. However, in the specimens used in this study, the zona pellucida had consistently been shed by the seventh day post ovulation and attachment along the anti-mesometrial surface was already underway in many places. This attachment was not reported by Mossman (1926) or Boving (1952) until the eighth day, a full day later than observed in this work.

Attachment on the antimesometrial surface beginning on the seventh day post ovulation is a result of the fusion and destruction of the uterine epithelium by localized, syncytial, thickenings of the trophoderm, the trophoblastic nodules. These nodules begin to appear by six days post ovulation and are numerous and prominent by the time the zona pellucida is shed. As the nodule and epithelium make positive contact, the cell membranes separating them break, forming a symplasm in which the trophoblastic material proceeds to destroy the epithelial cells. No general degeneration of the uterine epithelium into a syncytial mass was found as was reported by Mossman (1937) and Brambell (1951) and copied from Mossman into Amoroso's work of 1952. The only area in which a syncytium was found occurred in the places where the

trophoblastic nodule had destroyed the uterine epithelium. The remainder of the uterine epithelium was composed of perfectly normal, intact cells with no sign of degeneration.

The controlling factor in the orientation of the trophocyst in the uterus was described by Boving (1954) as resulting from the ability of the trophoblastic nodules of the abembryonic hemisphere to attach only to the epithelium of the antimesoendometrial surface. However, much doubt is cast on this by the fact that the trophocyst is continually oriented with the embryonic pole toward the mesoendometrial groove before the zona pellucida is shed.

By eight and one-half days post ovulation, the destructive forces of the trophoderm have reached such a high level that the entire endometrium, from the edges of the periendometrial ridges across the antimesoendometrial surface, along with the trophoderm and trophoblastic nodules has begun to disintegrate into a syncytial mass. This degeneration progresses as deep as the myometrium in many places but some of the deeper parts of the uterine glands remain to proliferate and regenerate a new uterine epithelium by the twelfth day. Giant Cells (Sansom, 1927; and Minot, 1890) form a conspicuous part of the sub-epithelial connective tissue. Degeneration of the original uterine epithelium and trophoderm has been described by Mossman (1937) and Brambell (1951) and the findings of this work support them up to this point. However, both Mossman (1937) and Brambell (1951) reported destruction of endoderm along with the trophoderm. In the specimens used in this study, the endodermal alyer remained intact at least three days after the destruction of the trophoderm, thus constituting the only layer of cells remaining of the abembryonic hemisphere of the embryonic vesicle during

this period. By the twelfth day post ovulation, the endoderm has dispersed and the yolk sac composes the abembryonic portion of the embryonic vesicle.

During the entire seventh day of gestation, the trophoderm over the mesoendometrial ridges was in a state of rapid proliferation, resulting in extreme thickening of the trophoderm. By the end of the seventh day, two distinct layers have been established; the cytotrophoderm, consisting of intact cells and constituting the inside layer next to the mesoderm; and the syncytiotrophoderm, with no internal cell membranes, nuclei packed together next to the cytotrophoderm, and the cytoplasm constituting a continuous, non-nucleated layer next to the epithelium. Similar thickening of the trophoderm in this area has been reported by Mossman (1937) but no reference could be found which described the division into cytotrophoderm and syncytiotrophoderm before attachment to the mesoendometrial ridges.

The uterine epithelium, during the seventh day has also undergone high proliferation until the layer has as many as six layers of nuclei in the pseudostratified columnar epithelium. The cells on the surface and in the glands have produced droplets which remain within the cell membrane, greatly distending the secreting cells. This was reported by Mossman (1926) but he considered the secretion to be extruded from the cell and added to the albumen layer.

Early in the eighth day, the thickened layers of trophoderm and uterine epithelium come in contact and the contacting cell membranes fuse. As on the antimesoendometrial surface, earlier workers, (Assheton, 1895; Mossman, 1926, 1937; Brambell, 1952; and Amoroso, 1952) have reported that "the

uterine epithelium swells and becomes syncytial as the trophoblast approaches it and is soon compressed into a thin layer between the trophoblast and its connective tissue. It then undergoes degeneration and disappears, apparently being absorbed by the cells of the trophoblast" (Amoroso, 1952). Nothing such as this was found in any of the specimens used in this study. The cells of the uterine epithelium were intact even after the outer cell membranes had fused with the membrane of the syncytiotrophoderm. Only after this fused membrane broke down and the contents of the syncytiotrophoderm mixed with that of the uterine epithelium are the inner cell membranes broken down and a symplasm formed. It becomes obvious that what earlier workers had described was a stage after the epithelium had broken down and been replaced by the syncytiotrophoderm and had mistaken the syncytiotrophoderm for a syncytial uterine epithelium and the cytotrophoderm for the entire trophoderm. This may also be the reason the split of trophoderm into cytotrophoderm and syncytiotrophoderm has not been reported previously.

After the uterine epithelium has been destroyed, pseudopodia-like projections of cytoplasm of the syncytiotrophoderm begin to extend into the connective tissue of the endometrium. This occurs during the latter part of the eighth day. These projections are not definite structures like villi but are irregular, soft plasmodia which move through the stratum spongiosum and uterine glands, destroying and absorbing maternal tissue in their path. Amoroso (1952) reported that the trophoderm invasion made use of the uterine glands as a "temporary pathway" and after destroying and replacing the glandular epithelium, forming the villi, proceeded out into the surrounding

connective tissue. Some of the plasmodial projections do extend into the uterine glands but as many, if not more, progress directly into the solid connective tissue. Those plasmodia extending into a gland seldom penetrate deeper than those penetrating solid tissue nor deeper than the level to which the connective tissue around them is being destroyed. Therefore, it is evident that the margin of destruction is a relatively straight line and that the tissue of the uterine glands is not destroyed any faster than that of the stratum spongiosum.

Behind the advance of the cytoplasmic plasmodia, the nuclei of the syncytiotrophoderm have become less compact and fill in the space once occupied by the maternal tissue which has been destroyed. Prcbably because of the close association with the blood, the endothelium of the blood vessels is not destroyed as quickly by the advancing syncytiotrophoderm. Thus, capillaries are bypassed by the cytoplasmic plasmodia and become surrounded by a layer of nuclei of the syncytiotrophoderm. The maternal endothelium is retained for a time but eventually disappears and maternal blood flows through a closed system of vessels of embryonic tissue. Similar replacement has been described by Grosser (1927), Mossman (1937), and Amoroso (1952) but their descriptions of the invasion process are void of any reference to a cytoplasmic invasion followed by a filling-in by the nuclei of the syncytiotrophoderm.

Behind the invasion of the syncytiotrophoderm, the cytotrophoderm starts during the ninth day <u>post ovulation</u> to become indented at regular intervals along its surface. These indentations are round tubes which

penetrate by an active proliferation of the cells at the base of the tube. They continue to elongate until by the twelfth day they have overtaken the advance of the syncytiotrophoderm, which has stopped just below the original uterine glands. These projections of cytotrophoderm, therefore, constitute the villi of the rabbit placenta and are similar to the uterine glands in appearance. However, they are completely independent of the uterine glands which were previously destroyed and the cells of which were absorbed before the villi ever started to grow.

As the cytotrophodermic villi extend into the placental pad, they in turn surround the blood channels and destroy the nuclei of the syncytiotrophoderm which had replaced the original endothelium, so that the maternal blood now flows through a system of channels composed of cytotrophoderm. In places a small part of the syncytiotrophoderm is retained as an endothelium-like lining.

It has been reported by Grosser (1927) and Mossman (1937) that one of the major characteristics of the rabbit placenta is that the ingrowth of trophoderm is not accompanied by embryonic mesoderm. The cytotrophodermal villi, from their very beginning, have a definite, heavy core of embryonic mesoderm. This core comes directly from the serosal mesoderm and distinctly fills the entire length of the villi. Grosser (1927) and Mossman (1937) also reported that it was only after the allantois has grown out from the embryo into the exocoel and has extended around the amnion and come into contact with the serosa that the placenta becomes vascularized. In contrast, in material used in this study, as early as the ninth day, the serosal mesoderm which

directly surrounds the base of the body stalk has become highly vascularized by allantoic blood vessels which extend through the body stalk although the allantois has not as yet extended from the body cavity. By the eleventh day, the entire placental pad has become vascularized by allantoic blood vessels and the allantois itself has still not extended from the body cavity.

Thus, the placental pad in the rabbit has been formed by a three front invasion: (1) cytoplasm of the syncytiotrophoderm which penetrates by pseudopodia-like projections and destroys the maternal tissue in its path; (2) nuclei of the syncytiotrophoderm, which follow behind the destruction of the cytoplasm and fill in the space once occupied by this tissue; and (3) villi of the cytotrophoderm, which penetrates the placental pad as undulating tubes, carrying mesoderm and embryonic blood vessels.

SUMMARY

In the study of placentation in the rabbit, entire uterine swellings of six to 25 days gestation were macroscopically and microscopically studied.

The nonpregnant rabbit uterus contains heavy mesoendometrial ridges aligned with the mesometrium and bordered by smaller periendometrial ridges. The surface opposite the mesometrium has small, irregular antimesoendometrial ridges. The endometrium of irregular connective tissue and columnar epithelium contains short, straight uterine glands.

The zona pellucida remains intact until the last half of the sixth day _post ovulation_ at which time it begins to be dissolved and by seven and one-half days _post ovulation_ it is completely gone.

Large, syncytial masses of trophoderm, the trophoblastic nodules, have begun forming over the abembryonic hemisphere by the sixth day. When these trophoblastic nodules come into contact with the uterine epithelium at six and one-half days, they fuse with and progressively destroy it down to the stratum spongiosum. No invasion was observed into the underlying connective tissue, and the epithelium in areas other than those involved in trophoblastic nodule invasion consists of intact cells. By seven and one-half days all of the trophoblastic nodules are attached and have destroyed a small portion of the epithelium. By the beginning of the eighth day of gestation the trophoderm of the abembryonic hemisphere has degenerated along with the entire anti-mesoendometrium except for some of the deepest portions of the uterine gland epithelium. The endoderm of the bilaminar omphalopleur, also degenerates after the twelfth day. The deep portions of the epithelium which remained intact proliferate and by the twelfth day post ovulation, completely reline the antimesometrial surface of the uterine lumen.

The trophoderm of the embryonic pole of the trophocyst, by seven and one-half days, has proliferated into a thick layer which is divided into an inner, cellular "cytotrophoderm" and a superficial layer of "syncytiotrophoderm." By the beginning of the eighth day, the syncytiotrophoderm fuses with the uterine epithelium and penetrates the mesoendometrial ridge by pseudopodia-like plasmodia destroying and absorbing maternal tissue as they go. During the last half of the eighth day the cytotrophoderm penetrates the syncytiotrophoderm with straight, tubular villus projections which carry a core of mesoderm. The trophodermal invasion thus takes place on two fronts; the

syncytiotrophoderm invading and destroying endometrium and the cytotrophoderm, in turn, invading and destroying syncytiotrophoderm.

By the twelfth day post ovulation, the villi of the cytotrophoderm, have overtaken and replaced all but a thin layer of the syncytiotrophoderm at the base of the placenta. The maternal capillaries have been surrounded and destroyed so that the maternal blood now flows through channels of cytotrophoderm. Allantoic blood vessels which have penetrated into the mesodermal cores of the villi of the placenta are thus brought into close proximity to the maternal blood channels until in the final state embryonic capillaries lie directly in channels of maternal blood.

It is the conclusion from this work that the rabbit trophocyst attaches itself on the antimesometrial surface of the uterus and by a two-front invasion of syncytiotrophoderm and cytotrophoderm, penetrates and destroys the tissue of the mesoendometrium, thus bringing the embryonic and maternal bloods into close proximity so that exchange can take place between the two systems.

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APPENDIX

EXPLANATION OF PLATE I

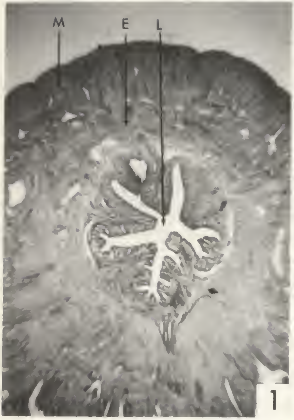
Fig. 1. Section of a nonpregnant rabbit uterus showing myometrium (M), endometrium (E), and uterine lumen (L). (35X)

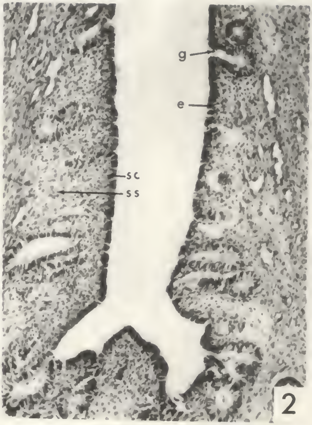
Fig. 2 Section of a nonpregnant rabbit uterus showing epithelium (e), uterine gland (g), stratum compactum (sc) and stratum spongiosum (ss). (100X)

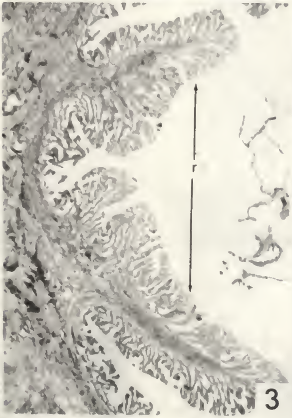
Fig. 3 Section of rabbit uterus at six days post ovulation showing enlarged mesoendometrial ridges (r). (50X)

Fig. 4 Section of a trophoblastic nodule showing the syncytial nature with the nuclei arranged around periphery and cytoplasmic center. (970X)

PLATE I







EXPLANATION OF PLATE II

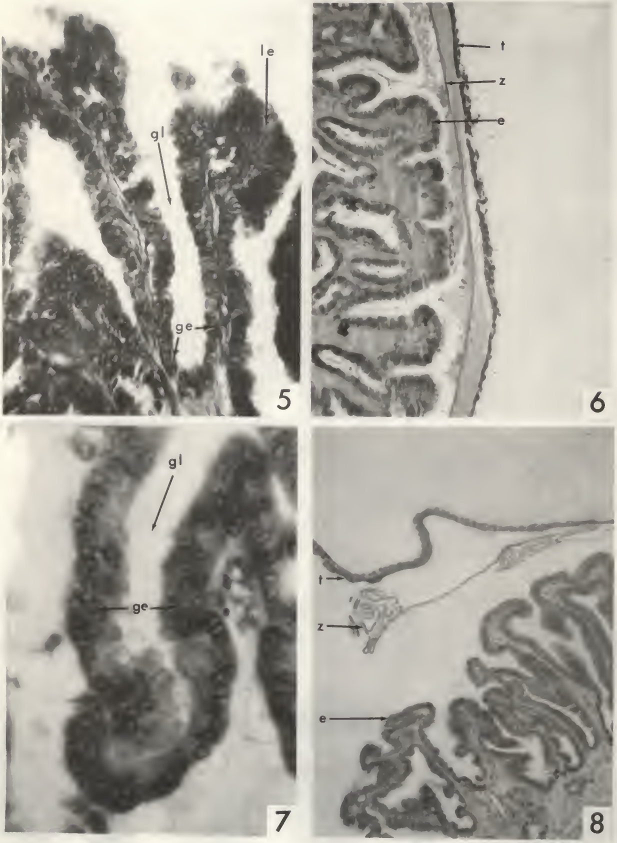
Fig. 5 Section through the mesoendometrial ridges at six and one-half days _post ovulation_ showing the thick, pseudocolumnar luminal epithelium (le), the cuboidal glandular epithelium (ge) lining the glandular lumen (gl). (430X)

Fig. 6 Section through the mesoendometrial ridges at six and one-half days _post ovulation_ showing the trophoderm (t) separated from the uterine epithelium (e) by the intact zona pellucida (z). (200X)

Fig. 7 Section through the mesoendometrial ridges at seven days _post ovulation_ showing the secretions of the glandular epithelium (ge) distending the cells until they close much of the area of the glandular lumen (gl). (970X)

Fig. 8 Section at seven days _post ovulation_ showing the zona pellucida (z) broken and convoluted, trophoderm (t) and uterine epithelium (e). (200X)

PLATE II



EXPLANATION OF PLATE III

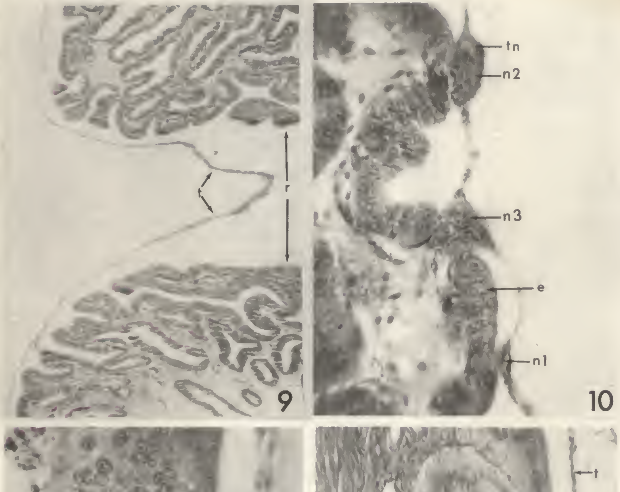
Fig. 9 Section through the mesoendometrial ridges (r) at seven days <u>post ovulation</u> showing no attachment of the trophoderm (t) to these ridges at this time. (35X)

Fig. 10 Section through the antimesometrial surface at seven days <u>post ovulation</u> showing the attachment of the trophoblastic nodules (tn) to the uterine epithelium (e). A very early attachment (n1) may be seen in which only an adhesion of the cell membranes has occurred as well as on intermediate stage (n2) in which the fused membranes have broken and a late stage (n3) in which the epithelial tissue has been almost completely destroyed. Note these attachments are not associated with the presence of maternal capillaries. (430X)

Fig. 11 Section through the trophoderm of the embryonic hemisphere at seven and one-half days <u>post ovulation</u> showing yolk sac (ys), cytotrophoderm (c) and syncytiotrophoderm (s). (750X)

Fig. 12 Section through the antimesometrial surface at eight days <u>post ovulation</u> showing the degenerating trophoderm (t), trophoblastic nodules (tn) and uterine epithelium (e). Below the line of destruction may be seen the glandular epithelium (ge) of the deeper uterine glands which remain intact. (200X)

PLATE III



EXPLANATION OF PLATE IV

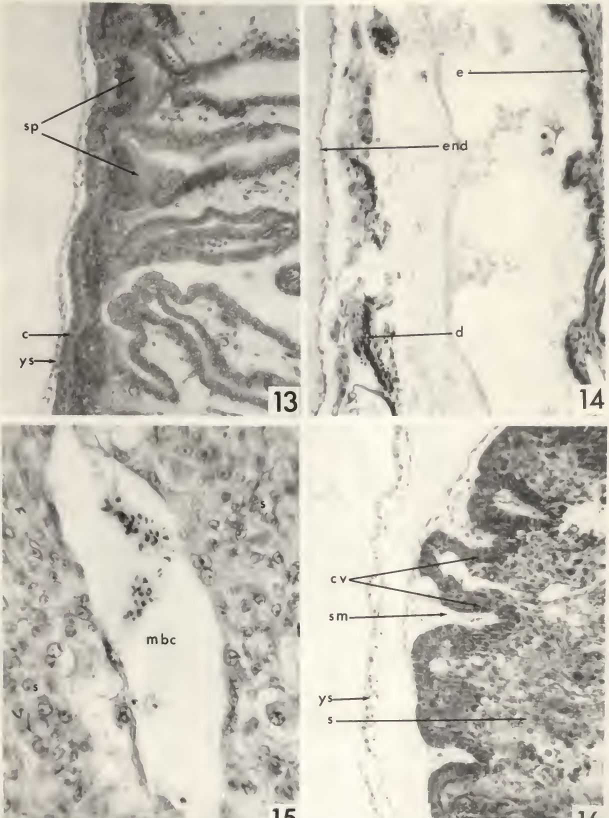
Fig. 13 Section through the mesoendometrial ridges at eight days post ovulation showing yolk sac (ys), cytotrophoderm (c) and early syncytiotrophodermal plasmodia (sp). (100X)

Fig. 14 Section through the antimesometrial surface at nine days post ovulation showing the debris (d) remaining from the destruction of the uterine epithelium and trophoderm, the intact endoderm (end), and the regenerating uterine epithelium (e). (100X)

Fig. 15 Section through the placental pad at nine days post ovulation showing a maternal blood channel (mbc) surrounded entirely by syncytiotrophoderm (s). (650X)

Fig. 16 Section through the placental pad at nine days post ovulation showing the syncytiotrophoderm (s) being penetrated by cytotrophodermal villi (cv) which have a definete core of serosal mesoderm (sm). Numerous embryonic blood vessels may be seen in the yolk sac (ys). (430X)

PLATE IV



EXPLANATION OF PLATE V

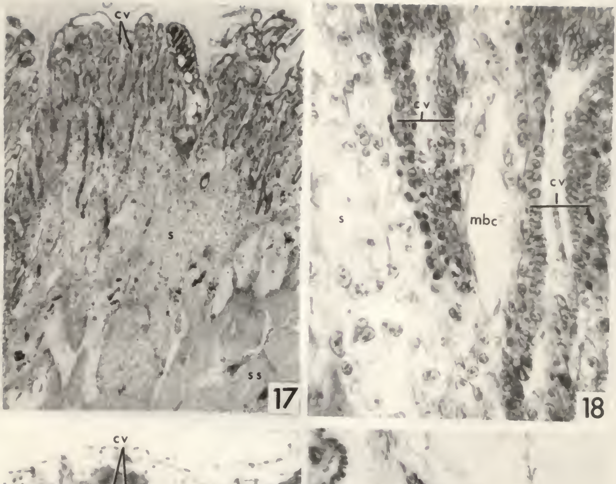
Fig. 17 Section through the placental pad at ten days post ovulation showing stratum spongiosum (ss) which has not been destroyed, syncytiotrophoderm (s) which is invading maternal tissue and cytotrophodermal villi (cv) which is in turn invading and destroying syncytiotrophoderm. (35X)

Fig. 18 Section through the placental pad at ten days post ovulation showing syncytiotrophoderm (s) being penetrated by cytotrophoderm (cv), the latter surrounding a maternal blood channel (mbc) so that the maternal blood now flows through cytotrophodermal tissue. (650X)

Fig. 19 Section through the placental pad at ten days post ovulation showing cytotrophodermal villi (cv) which have cores of serosal mesoderm (sm) which have become highly vascularized by allantoic blood vessels. The latter may be recognized by containing many embryonic erythrocytes which show a dark staining nucleus. (100X)

Fig. 20 Section through the antimesometrial surface at 12 days post ovulation showing the regenerated uterine epithelium (e), the giant cells (gc) and the degenerating endoderm (end). (100X)

PLATE V



EXPLANATION OF PLATE VI

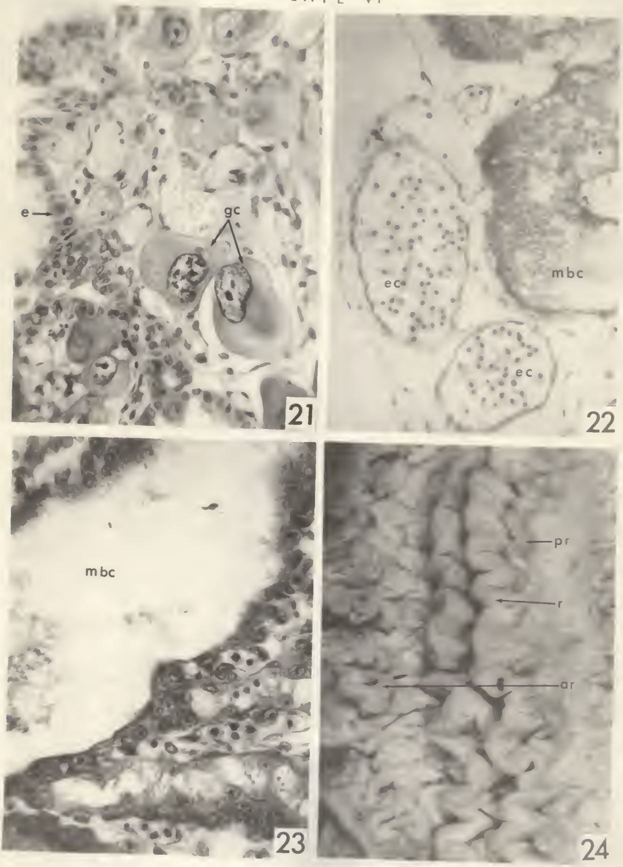
Fig. 21 Section through the antimesometrial surface at 12 days _post ovulation_ showing giant cells (gc) lying beneath the new uterine epithelium (e). (750X)

Fig. 22 Sections through the surface of placental pad showing embryonic capillaries (ec) in close proximity to maternal blood channels (mbc). Again the embryonic capillaries are easily recognized by the dense nuclei of the embryonic erythrocytes. (430X)

Fig. 23 Another section as Fig. 22. (650X)

Fig. 24 Gross view of nonpregnant rabbit uterus which has been opened longitudinally along the antimesometrial surface showing the mesoendometrial ridges (r), the periendometrial ridges (pr) and the irregular antimesoendometrial ridges (ar). (10X)

PLATE VI



PLACENTATION IN THE RABBIT

by

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B. A., William Jewell College
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requirements for the degree

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Manhattan, Kansas

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ABSTRACT

Entire domestic rabbit uterine swellings containing embryos and placentae were collected at timed stages, fixed, serially sectioned, stained and studied. The series covered the range of six to 25 days post ovulation.

The uterus of the rabbit contains a pair of heavy "mesoendometrial ridges," extending the length of the uterine horn on the surface corresponding to the attachment of the mesometrium. These are bordered on the outer side by two smaller "periendometrial ridges" and the remainder of the wall exhibits irregular, inconsistent antimesoendometrial ridges. The uterine epithelium is columnar over the surface and extends into the lumina of the short, straight uterine glands.

The zona pellucida, which has surrounded the conceptus from fertilization, remains intact until the last half of the sixth day post ovulation at which time it begins to be broken or dissolved at different spots over the abembryonic portion of the trophocyst. By seven and one-half days post ovulation the zona is completely gone.

Large, syncytial masses of trophoderm, the trophoblastic nodules, have begun forming over the abembryonic hemisphere by the sixth day and are numerous by the time the zona pellucida is broken. When these trophoblastic nodules come into contact with the uterine epithelium, they fuse with and progressively destroy it down to the stratum spongiosum. No invasion was observed into the underlying connective tissue, and the epithelium in areas other than those involved in trophoblastic nodule invasion consists of intact cells.

First attachment of trophoderm to endometrium occurred in the last half of the sixth day. By seven and one-half days all of the trophoblastic nodules are attached and have destroyed a small portion of the epithelium. By the beginning of the eighth day of gestation the trophoderm of the abembryonic hemisphere has degenerated along with the entire antimesoendometrium except for some of the deepest portions of the uterine gland epithelium which lie along the myometrium. The endoderm of the bilaminar omphalopleur, also degenerates after the twelfth day. The deep portions of the epithelium which remained intact proliferate and by the twelfth day _post ovulation_, completely reline the antimesometrial surface of the uterine lumen.

The trophoderm of the embryonic pole of the trophocyst, by seven and one-half days, has proliferated into a thick layer which is divided into an inner, cellular "cytotrophoderm" and a superficial layer of "syncytiotrophoderm." By the beginning of the eighth day, the syncytiotrophoderm fuses with the uterine epithelium and penetrates the mesoendometrial ridge by pseudopodia-like plasmodia destroying and absorbing maternal tissue as they go. During the last half of the eighth day the cytotrophoderm penetrates the syncytiotrophoderm with straight, tubular villus projections which carry a core of mesoderm. The trophodermal invasion thus takes place on two fronts; the syncytiotrophoderm invading and destroying endometrium and the cytotrophoderm, in turn, invading and destroying syncytiotrophoderm.

By the twelfth day _post ovulation_, the villi of the cytotrophoderm, have overtaken and replaced all but a thin layer of the syncytiotrophoderm at the base of the placenta. The maternal capillaries have been surrounded and

destroyed so that the maternal blood now flows through channels of cytotrophoderm. Allantoic blood vessels which have penetrated into the mesodermal cores of the villi of the placenta are thus brought into close proximity to the maternal blood channels until in the final state embryonic capillaries lie directly in channels of maternal blood.

It is the conclusion from this work that the rabbit trophocyst attaches itself on the antimesometrial surface of the uterus and by a two-front invasion of syncytiotrophoderm and cytotrophoderm, penetrates and destroys the tissue of the mesoendometrium, thus bringing the embryonic and maternal bloods into close proximity so that exchange can take place between the two systems.